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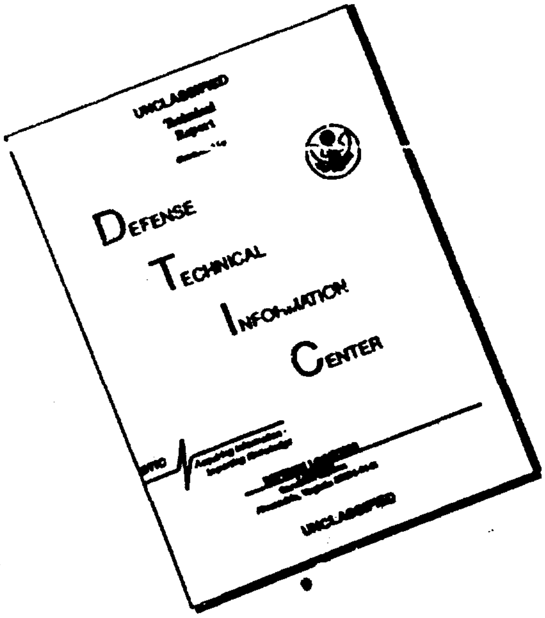

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SANITARY AND BACTERIOLOGICAL METHODS AND THE SOLUTION OF PRACTICAL SANITARY PROBLEMS

[Following is a translation of an article by Professor L.I. Mats from the Institute of General and Communal Hygiene imeni A.N. Sysin of the Academy of Medical Sciences of the USSR in the Russian-language periodical <u>Gigiyena i Sanitariya</u> (Hygiene and Sanitation), No 10., Moscow, 1962, pages 94-100. The article was submitted for publication on 28 February 1962.]

In recent years there have been a number of articles in the magazine <u>Gigiyena i Sanitariya</u> on methodological questions of sanitary microbiology (1960-1961). The magazine has properly designated these methods for discussion, because many of them, without any need, require laborious efforts from the practical laboratories.

A substantial place in sanitary microbiology, as in science, is given to the development of methods of evaluating the sanitary-hygienic state of objects in the external environment and to the indication of pathogens in the same environment.

It is obvious that in solving their basic problems the scientific workers in the field of sanitary microbiology can employ any method which will enable them to better solve the given problem. However, the methods

which are proposed for wide usage in sanitary-epidemiological laboratories for evaluating the sanitary-hygienic and epidemiological condition of objects in the external environment should be very simple. The purpose of sanitary analysis, to include the microbiological, is to place in the hands of the sanitary doctor objective data on the sanitary-hygienic condition of an object in order for him quickly to understand the sanitary situation and to conduct the appropriate preventative, sanitary, and other measures.

The early detection of a pathogen in the external environment is an important link in the struggle to lower the incidence of infectious diseases.

The analysis and the method of conducting it should be very precise, rapid to perform, suitable for any situation, and economical in cost. From the point of view of the sanitary-microbiological and epidemiological characteristics of objects of the external environment, it is important to investigate the greater part of the specimens using simple methods rather than to investigate chance specimens using more complex methods, because the concentration of sanitary-indicator microorganisms and pathogens of infectious diseases and even their accumulation in specimens in the external environment are comparatively small.

The microbiological methods of indicating pathogenic organisms which are ordinarily employed require a long time to be performed. The small concentration of pathogenic microorganisms in the external environment and the abundance of saprophytes and to a considerable degree of antagonists makes it difficult

to detect pathogenic microorganisms in the air, water, and soil. As a consequence of this, in the practice of sanitary microbiology the direct determination of pathogenic bacteria is rather frequently replaced by the search for so-called sanitary-indicator microorganisms (colibacilli, streptococci, staphylococci, bacteriophages, etc.).

The sanitary-indicator microorganisms (for example, varieties of colibacilli) are generally sufficiently delicate indicators of the sanitary-hygienic condition and especially of the processes of atrophy of bacteria in the external environment (water, soil, food products, etc). However, they cannot replace or search for pathogenic microorganisms as an epidemiological evaluation of the external environment and not all of the proposed methods and sanitary-indicator microorganisms are of equal value in the sanitary evaluation of specimens from the external environment.

It should be noted that the determination of sanitary-indicator microorganisms in the air gives answers which are very tardy for preventative and positive sanitary measures. All atempts to obtain general indicators of the sanitary-hygienic condition of the air using bacteriological test data have borne a chance character and the indicators themselves have a very relative significance.

The sanitary-indicator value of determining the total number of bacteria and strepto-staphylococci as indicators of the sanitary condition of the air of living quarters is very small. The determination of the strepto-staphylococci under practical conditions

in laboratories of sanitary-epidemiological stations is rather complex, cumbersome, and long. The investigation of the sanitary-indicator value of strepto-staphylococci in the air does not have the same meaning as does the investigation of the sanitary-indicator value of the colibacillus in water. In testing water for the presence of the colibacillus one has in mind not only the indirect determination of the epidemiological state of the reservoir but also the hygienic study of the dynamics of the processes of self-purification and the inhibition of the bacteria in the water of the reservoir which is very important in evaluating the sanitary state of the latter.

The considerable mobility of the aeroplankton means that the analysis establishes the state of the microflora of the atmospheric environment only at one certain point, whereas this state of the microflora changes rapidly.

Bacteriological analysis gives a high percentage of deviations, a tardy answer, is expensive, and using it, it is not possible to obtain firm data for accurate conclusions. This is understood by the researchers who propose various norms for the content of micro-organisms in the air.

The norms are given only for unventilated living quarters. However, unventilated living quarters are impermissible from a sanitary-hygienic point of view and should be so considered without a bacteriological test.

At the Fourteenth All-Union Congress of Hygienists and Sanitary Doctors in 1961, Professor V.A. Ryazanov,

a corresponding member of the Academy of Medical Sciences of the USSR, properly pointed out that the study of the bacteriological contamination of the air does not justify itself in evaluating the sanitary-hygienic state of the air.

In ordinary practice the analysis of the aeroplankton should be used only in special cases (in accordance with the order of the Ministry of Public Health of the USSR during the systematic observation of maternity homes, surgical operations, the production of bacterial preparations and sterile medicines, etc.) and in accordance with epidemiological indications. Only the direct immediate determination of pathogenic microorganisms in the air is of definite importance.

In the field of theoretical aeromicrobiology the first problem which comes to mind is the development of methods for the direct and rapid indication of pathogenic bacteria and viruses, especially the flu virus and other adenoviruses and Rickettsia (Rickettsia burneti, Q fever).

The Soviet standard establishes the determination of all varieties of the Bacillus coli as an indicator of the sanitary-hygienic state of the soil, water, and food products.

There is a considerable number of varieties of the Bacillus coli, because various representatives of this group of bacteria differ with respect to the assimilation of different sources of carbon and nitrogen. The Bacillus coli has a polyantigenal structure. A considerable number of serological varieties are encountered. All the biochemical and serological varie-

ties of the Bacillus coli under certain conditions can cause various pathological occurrences (infections, food infections and intoxications, etc.) in people, especially in children and in a weakened organism; these pathological phenomena depend on the immunobiological state of the macroorganism.

Professor V.M. Zhdanov, an active member of the Academy of Medical Sciences of the USSR, spoke at the Fourteenth All-Union Congress of Hygienists and Sanitary Doctors and pointed out the great importance of the immunological state of the organism when subjected to the pathological processes caused by the Bacillus coli.

Under ordinary conditions the Bacillus coli is a saprophyte because throughout the long course of history people in general developed immunity to this microbe.

The study of the dynamics of the existence of the Bacillus coli in a water reservoir is a good indication of the sanitary state of the water source and a check on the quality of the purification of drinking water. Numerous investigations of different reservoirs in our country which were conducted in the 1930's under the direction of the Central Institute of Communal Hygiene (Professor S.N. Stroganov) showed that the coli titer was not less than 0.3 at points where there was completed self-purification of the reservoir.

The considerable research performed in the Department of Communal Hygiene of the First Moscow Medical Institute (corresponding member of the Academy of Medical Sciences of the USSR, S.N. Cherkinskiy) demonstrated that a coli index for drinking water of not greater than 3, which was accepted by the Soviet-

All-Union State Standard 2874-54 as the norm indicator of the quality of water with respect to bacteriological can be considered as a very dependable indicator of the effectiveness of the purification of water in cases where it has been contaminated with the pathogens of intestinal infections and also of brucellosis and leptospirosis.

It is generally known that the beginning of the decline in the incidence of typhoid fever in inhabited points of Europe and the USA coincided in time with the conduct of measures to prevent the contamination of the soil by waste (planning and organisation of public services, purification, sewer systems, etc.) and with the supplying of good-quality drinking water (with a Bacillus coli content of 100 or less per liter of treated water) to the population. After the implementation of these measures the death rate from typhoid fever in England, which had been 475 persons out of one million up to the 1890's, began to drop in the middle of the 1890's when they began to provide public services in the towns and to supply good quality water. By 1910 the mortality rate had dropped to 50 persons out of one million. This indicates the great importance of bacteriological analysis in checking on the operation of various water-purifying and disinfecting (chlorinating) units for water systems. In such cases bacteriological analysis is a dependable indicator which makes it possible to judge the degree of purification and disinfection. The slightest disruption of the operation of purification and disinfection equipment has an immediate effect on the quantitative and

qualitative microbe composition of the water being treated.

It is not necessary to complicate the determination of fecal contamination by additional research into other fecal microbes: the fecal streptococci and Clostridium Welchii (Bac. perfringes Welchii). The group of streptococci is found rather widely in the external environment; for a precise identification of the fecal streptococcus it is necessary to study deeply the biochemical and serological characteristics of the microbe, which is a substantial delaying factor.

There is a significant difference between the characteristics of the Bacillus coli and the Clostridium Welchii. In the case of fresh contamination, objects in the external environment simultaneously receive bacteria of the groups Bacillus coli and Clostridium Welchii. The Bacillus coli does not form a spore and perishes rather quickly in the external environment; therefore, its presence can indicate more or less fresh contamination. The Clostridium Welchii forms spores and is preserved for a long time in an object of the external environment. As a consequence, relatively recent contaminations of objects of the external environment can contain Clostridium Welchii but may also be free of the Bacillus coli.

M.I. Tarkov (Moldavian Institute of Epidemiology, Microbiology, and Hygiene, 1961) presented convincing data on the possibility of the multiplication of the Clostridium Welchii in the soil under certain conditions. The question arises of the necessity of reexamining the possibility of considering the Clostridium Welchii as

an indicator of even old fecal contamination.

Professor G.P. Kalina (1960) with some basis criticized the so-called temperature test, i.e., the capacity of a variety of the Bacillus coli for gas formation at 43 degrees Centigrade in liquid media with mannitol or glucose; this test is the basic sign of the sanitary-indicator value of the given variety of Bacillus coli.

I.S. Minkevich considered that this is the most characteristic sign indicating the origin of the microbes from the feces of man or warm-blooded animals. The Bacillus coli in cold-blooded animals does not ferment glucose or mannitol in liquid media at this temperature. But G.L. Zmeyev (1944) and I.S. Minkevich himself (1949) considered that for the southern regions of the Soviet Union (Central Asia) this test is not an indicator for the Bacillus coli from the feces of man or warm-blooded animals because the Bacillus coli in cold-blooded animals of the South of the USSR is able to ferment glucose and mannitol at 43 degrees Centigrade.

The growth of large inhabited points and the considerable contamination of adjacent rivers by them doubtlessly is causing sharp changes in the composition of the varieties of Bacillus coli in the feces of both man and of warm and cold-blooded animals. Further research in this direction is required.

Let us say a few words about the two-phase method of testing household-drinking water for the Bacillus coli as recommended by the All-Union State Standard 5216(56). Up to the present time this method has

encountered (without any basis) objections from a number of sanitary bacteriologists. At the same time the two-phase method of detecting the Bacillus coli in drinking water is considerably more advanced than the old three-phase method because it shortens the time for the analysis to 24 hours, is easy to perform, especially under field conditions, can be done successfully by a laboratory technician with a secondary medical or biological education, and is no less accurate than the three-phase method. In a small number of cases the two-phase method can sometimes give a small increase in the number of Bacillus coli at the expense of other gram negative bacteria such as the cloaco bacillus, Proteus, Bacillus pyocyaneus, and other bacilli. However, this does not interfere particularly with sanitary supervision because it only leads to somewhat higher demands in processing household-drinking water.

Different authors have established that bacteriophages are detected regularly in drain water from an open reservoir in the area of an inhabited point.

M.N. Fisher and his coworkers (1955) note the relationship between the content of dysentery bacteriophages in the water, its coli titer, and the isolation of dysentery haptene from it. As a result of their research, I.I. Goriyenko and K.F. Goncharova (1961) established a certain parallelism between the frequency of finding a bacteriophage in the water, the coli titer, and the general intake of bacteria. The authors note that most of the secreted phages possessed a strict species specificity but with a broad range of action with a species. Some of these phages were not strictly speci-

fic, possessed polyvalent characteristics, and dissolved some species of bacteria. However, despite the considerable number of works on this problem, tests of objects of the external environment for the presence of phages has not been accepted in the practice of sanitary-bacteriological research.

Detecting phages does not have any advantages in comparison with the titer of Bacillus coli; and the conduct of the analysis is considerably more complex than the widely proven detection of the Bacillus coli. In epidemiological practice the detection of phages cannot give definite results because of the above-mentioned polyvalence of various phages. In recent times direct and specific determination of various pathogens of infectious diseases in objects of the external environment by determining the increase of the titer of a specific phage has been introduced widely in epidemiology (V.D. Timakov and D.M. Gol'dfarb, 1960).

For the reaction of the increase of the titer of the phage it is necessary to have phages with a rigidly fixed range of action and with known characteristics.

The occurrence of various saprophytes, including the Bacillus coli, in food products and all kinds of washings for which there are no corresponding standards on bacterial data (except preserves) cannot serve as the basis for their rejection because the spoiling of the products depends not only on the quality of the saprophytes but also on their quantity. The progressive spoiling of a product is established by simple organo-

leptic determinations. The analysis of individual products sent to a laboratory by sanitary control with a request only to determine the quantity of microbes and the titer of the Bacillus coli usually does not give any basis for establishing whether or not the products are fit for consumption. For this it is necessary, in addition to the bacteriological analysis, to make organoleptic and chemical tests of the food product. And one organoleptic determination can often serve as sufficient basis for condemning products.

The content of the various saprophytes, including the Bacillus coli in considerable quantities, can serve as only a relative indicator of the quality of the product and, in particular, of the sanitary conditions of the preparation, transportation, and storage of the product; therefore, prophylactic bacteriological analysis of a food product should be conducted when there is constant observation of the production processes, as in a bakery, food plant, etc.

The so-called mixtures and dairy products intended for children are subject to especially careful bacteriological analysis. For these products which undergo special thermal processing or are prepared with special fermenting cultures, the determination of the Bacillus coli and of the saprophytes gives the sanitary doctor the data for a sanitary evaluation of the product.

The discovery in food products of pathogenic bacteria, including the pathogens of food infections and intoxication, and also of viruses serves as a direct indicator for forbidding the use of the product as food

without appropriate processing as determined for each separate case.

In performing prophylactic measures it is sufficient to determine only the species of the pathogen. When further study is necessary, the determination of the antigenal structure and the serological type and phage type of the isolated pathogen, including the Salmonella, should be conducted only at well-equipped oblast and republic sanitary-epidemiological stations or even at institutes.

If the practical laboratories of the sanitary-epidemiological stations are freed of this laborious and inappropriate and sometimes even useless work, they will be able to discharge their direct responsibilities more effectively.

A task for theoretical sanitary microbiology is to develop accelerated methods for detecting pathogenic microorganisms in the external environment. The development of the detection of enteroviruses (poliomyelitis virus, coxa virus, in soil, water, and food products is of particular interest. This has been studied only slightly.

Modern achievements in the fields of physics (optics) and biology (preparation of elective media, the serology and utilization of antibiotics, antagonists, and methods of concentrating bacteria) have opened sufficiently broad perspectives for the sanitary microbiologists for developing direct and accelerated methods of detecting pathogenic microorganisms in the external environment.

In recent years the identification of bacteria

using microscopy has broadened considerably, especially with respect to ultraviolet and infrared radiation.

Microscopic objects, including microorganisms, absorb and scatter light waves of a certain wave length, i.e., they possess definite absorptive and diffusing capabilities with respect to light waves of a certain wave length. By selecting the appropriate light filter it is possible to measure the absorptive or diffusing capability of microorganisms with respect to light waves.

The absorption and diffusion spectra for light are peculiar for each species of microorganism and can be used for determining the species and the quantity. By linking for the microscopic study of objects the appropriate light filters, photosensitive elements and electronic photoenlargers and also an oscillograph or television apparatus, it is possible to obtain an image of microorganisms (their morphology, quantity, etc.) on the screen of the oscillograph or television set which is visible to the naked eye (Kidle, 1956; Basis, Marcel, 1959).

In the USSR A.Ye. Mikirov (1957) has been working on the detection of microparticles in precipitation and clouds and in the solid and liquid fractions of aerosols through the study of their optical characteristics on the basis of studying their spectrum.

By selecting the appropriate light filters, A.Ye. Mikirov considers it possible to make a rapid determination and calculation of microorganisms in the external environment. According to the data of A.Ye. Mikirov, L.I. Mats, and Ye.Yu. Lebedeva, it is possible

using a gaseous light filter to detect 8% of the yeast cells which are dispersed in the air.

The analysis of the problems of employing the optical properties of microorganisms together with microscopy, photography, and television for the rapid indication of microorganisms in the external environment (air, water, etc.) offers extraordinary possibilities.

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